DECLARATION UNDER RULE 132		
	Application #	10/553,801
	Confirmation #	6107
	Filing Date	03/25/2008
	First Inventor	PUZO Germain
	Art Unit	1623
	Examiner	Krishnan, Ganapathy
	Docket #	0512-1404

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Germain PUZO, residing at 2 Impasse Goudouli, 31320 Auzeville Tolosane, France, declare and say as:

1. I am a French citizen.

2. I obtained a phD in Biochemistry from University Paul Sabatier (Toulouse) in 1978. I am the author of more than 120 scientific publications in the field of mass spectrometry and mycobacterial and head of the group "Immunochemistry and mycobacterial glycoconjugates" at the Institute of Pharmacology and structural Biology.

3. I am an inventor of the above-identified application, and I am aware that claims 38-40 of the present patent application are rejected under 35 U.S.C. 112 as the specification would not provide sufficient enablement for the prophylaxis of tuberculosis.

4. Claims 38-40 provide for the use of the sulfoglycolipids of the invention in the treatment or prophylaxis of tuberculosis.

5. Example 3 of the specification as filed (attached herewith) is an *ex vivo* assay of the sulfoglycolipids of the invention characterizing the immunogenicity of the sulfoglycolipids. The results show that:

- sulfoglycolipids stimulate the cells of the immune system (abT-cells CD1 restricted);
- the sulfoglycolipids-reactive T-cells clones are specific for the sulfoglycolipids;
- the sulfoglycolipids-responsive T cells produce an antibacterial molecule (granulysin);

- the secretion of granulysin kill the pathogen (*M. tuberculosis*) and hence eradicates the infection;

- this response is specific for the PPD^+ population (patients which are positive to the tuberculin test and hence possibly infected with *M. tuberculosis*) as the PPD^- population (negative to the tuberculin test) does not elicit this response.

Altogether, these results show that the sulfoglycolipids do not act as an antibiotic in that they do not have a direct effect by killing the bacteria.

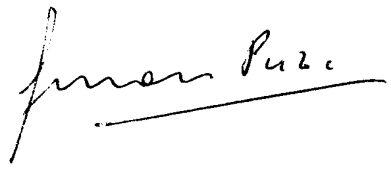
On the contrary, they stimulate a specific sulfoglycolipid cytotoxic T cell subpopulation which in turn via the production of granulysin will kill the bacteria, said response being specific in patients which are in contact with the bacteria.

This function thus allows the prophylaxis of the tuberculosis in that it has only an effect on the populations which are (or would be otherwise) infected by *Mycobacterium tuberculosis*.

6. The prophylaxis of tuberculosis is thus sufficiently enabled by the specification as filed.

7. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 19th September 2011



Germain PUZO

Encl.: Ex. 3 of the specification as filed

ANNEX

Example 3

***Ex vivo* assay of *Mycobacterium tuberculosis* sulfoglycolipids**

To define the immunogenicity of sulfoglycolipids-antigens, IFN-g release was measured after stimulation with the purified sulfoglycolipids. 2 x 10^5 PBMC per/well were incubated for 4 days in the presence of GM-CSF (500 U/ml) and IL-4 (5 ng/ml). Autologous effector T-cells were incubated in 10% human serum during this time. The sulfoglycolipids (10 µg/ml) were added to the irradiated, CD1-expressing antigen-presenting cells. Finally, effector cells were added (2 x 10^5/well) and IFN-g release was measured by ELISA in the supernatants after 18 hours. The IFN-g ELISA was performed in 96-well immunosorbent plates, which were coated with an IFN-g capture antibody (2 µg/ml) overnight. Non-specific binding sites were blocked with PBS containing 1% bovine serum albumin. The supernatants were diluted 1:1 and added in a final volume of 100 µl. Plates were incubated at room temperature for 2 hours and removed by thorough washing (3-4 times). Finally, a biotinylated anti-IFN-g antibody was added for 1 hour (2 µg/ml). For detection of immunoreactive IFN-g, horseradish-peroxidase was added for 30 min. Finally, a chromogenic substrate (TMB, Endogen, MA, USA) was added. After 20 min. incubation the reaction was stopped by the addition of sulfuric acid (2%). The intensity of the staining was determined photometrically at a wavelength of 480 nm. To estimate the concentration of cytokine in the supernatants, an IFN-g standard with a known concentration was included in all tests. 82 PPD^+ donors (positive to the tuberculin test) and 54 PPD^- donors (negative to the tuberculin test) were recruited and the response to the sulfoglycolipids was measured. Of the 82 PPD^+ donors 46 (56%) produced IFN-g (range 72 to 798 pg/ml, sensitivity of the ELISA test: 15 pg/ml), as compared to only 4 control patients (7%).

To characterize the function of T cell clones, which recognize sulfoglycolipids, the expression of antibacterial effector molecules was measured and the antibacterial activity of dendritic cells (DC) infected with virulent *M. tuberculosis* was determined. To detect granulysin, sulfoglycolipids-reactive T cell clones were permeabilised using 0.5% saponin and a polyclonal rabbit serum directed against granulysin was added. Staining was visualized using a FITC-conjugated secondary antibody directed against rabbit immunoglobulins. The number of positive cells was quantitated by analyzing cells in a flow cytometer.

For measuring the killing of *M. tuberculosis*, DCs were generated from peripheral blood monocytes by treatment with GM-CSF and IL-4. The cells were then infected with a virulent strain of *M. tuberculosis* (H37Rv) at a multiplicity of infection of 1. Non-phagocytosed bacteria were removed by thorough washing and then T-cells were added in increasing

amounts as indicated in **Figure 4**. The number of surviving bacteria was determined by plating cell lysates after 5 days of coincubation and counting the number of colonies grown after three weeks. Thus, it has been shown that T-cells specific for sulfoglycolipids recognize cells infected with live mycobacteria and - most importantly - kill the pathogen. Since sulfoglycolipids-responsive cells express granulysin, the secretion of this antibacterial molecule is most likely responsible for the killing of the pathogen. Therefore, sulfoglycolipids are presented on the surface of human host cells for mycobacteria recognition and killing.

Hence, a biochemically distinct and well characterized mycobacterial lipid antigen that is predominantly recognized by PPD^+ donors, but not by naïve controls, was identified. T-cell clones specific for sulfoglycolipids recognize DC infected with live *M. tuberculosis* bacilli and kill the pathogen possibly by the secretion of granulysin.